

Amendments to the Specification:

Please add the following new paragraph after the title RECOMBINANT MINIMAL CATALYTIC VANADIUM HALOPEROXIDASES AND THEIR USES on page 2:

--RELATED APPLICATIONS

This application is a division of U.S. Patent Application No. 09/596,794, filed June 19, 2000. This application is also related to U.S. Patent Application No. 09/151,189, filed September 10, 1998, now U.S. Patent No. 6,232,457, the disclosure of which is incorporated by reference in the entirety.--

Please replace the paragraph beginning at page 13, line 14 with the following:

-- Figure 5 shows motifs conserved among algal and fungal haloperoxidases (SEQ ID NOS:9, 12 and 13, 10, 14-16 and 11, and 17-19, respectively).--

Please replace the paragraph beginning at page 23, line 1 with the following:

-- DNA Hybridization Method. Hybridization probes were prepared at the second and near the third regions shown to be conserved between *Curvularia* and *Ascophyllum* vanadium peroxidase active sites by Messerschmidt, *et al.*, *PNAS*, 93:392-396 (1996). Hybridization probes of 51 base pairs were designed with Oligo 5.0 Primer Analysis Software (National Biochemicals, Plymouth, MN), synthesized by Anagen (Palo Alto), and digoxigenin-labeled at the 5' end with the Genius system (BMB Biochemicals, Durham, NC). The sequence of the probe for the second conserved site was:

CCAACGCACCCTTCGTACCCGTCTGGCCACGCTACCCAAAACGGAGCATTT (SEQ ID NO:3).

The sequence of the probe for the third conserved site was:

CCGTACGAACACTTCACCAGGAGCTGATGACTTTCGCCGAGGAATCCACCT (SEQ ID NO:4).--

Please replace the paragraph beginning at page 24, line 3 with the following:

-- The following Fucus peroxidase LIC primers were designed with Oligo software (National Biosciences, Inc., Plymouth, MN) and pET-32 LIC sequences necessary for ~~incorporated~~ incorporation into the vector-(normal font). Primers for the 5' end were: GACGACGACAAGATGCTTTGCCATGCAGCGGACA (SEQ ID NO:5) (34 bp) for the full length construct, GACGACGACAAGATGGCGCCGAATAGAAGGGACAA (SEQ ID NO:6) (35 bp) for the mid length construct, and GACGACGACAAGATGCTCTTCCGAGCGACCTTC (SEQ ID NO:7) (33 bp) for the short construct. One 3'-primer, GAGGAGAAGCCCGGTTGCACTAAGCCTGGCAGT (SEQ ID NO:8) (33 bp) was used for all three constructs. PCR was carried out for 30 cycles of 3 min at 94°, 1.5 min at 55° C and 2.3 min at 72° C, in 7 mM MgSO₄ for the full length construct and 4 mM MgSO₄ for the two truncated constructs. The PCR products were electrophoresed in 1.5% agarose and stained with ethidium bromide. DNA was extracted from the excised bands in GenElute minus EtBr spin columns (Supelco, Bellefonte, PA) and precipitated with ethanol.--

Please replace the paragraph beginning at page 25, line 5 with the following:

-- The recombinant bromoperoxidase proteins were immediately purified from bacterial cytoplasmic proteins by affinity chromatography. The HisTag (a sequence of 6 histidines, 6xHis (SEQ ID NO:20)) in the fusion protein was bound to a nickel nitriloacetic acid (Ni²⁺-NTA) agarose column (Sigma, St. Louis, MO) according to the standard Qiagen protocol. The bound recombinant protein containing 6xHis (SEQ ID NO:20) was eluted with 1M imidazole in 20 mM Tris-HCl pH 7.9 and 500 mM NaCl. Denatured protein samples were electrophoresed in 8% polyacrylamide gels containing 4% SDS, fixed and stained with Coomassie Brilliant Blue R250. The recombinant vanadium peroxidases (rVPx) were tested for

vanadium-dependent peroxidase activity on dot blots. A preliminary in vitro expression experiment was carried out for the three LIC constructs in a bacteriophage transcription system linked with a rabbit reticulocyte translation system (Single Tube Protein System 2, T7, Novagen), and the products were tested for vanadium-dependent peroxidase activity on dot blots.--

Please replace the paragraph beginning at page 28, line 1 with the following:

-- The *Fucus* sequence contains three conserved vanadium-binding regions (Messerschmidt *et al.*). The three conserved vanadium-binding regions are as follows: (1) amino acids 452-473 -AQRASCYQKWQVHRFARPEALG (SEQ ID NO:9); (2) amino acids 528-546 -PTHPSYPSGHATQNGAFAT (SEQ ID NO:10) and (3) amino acids 591-609 NKLAVNVAFGRQMLGIHYRFD (SEQ ID NO:11). -In the three conserved vanadium-binding regions the *Fucus* and *Ascophyllum* amino acid sequences differ only at two locations in the first conserved region (alanine at *Fucus* 455 substituted for serine at *Ascophyllum* 19, and cysteine at *Fucus* 457 substituted for tryptophan at *Ascophyllum* 21). These two amino acid differences are therefore likely to be related to the greater specific activity of the *Fucus* enzyme, as are other amino acid sequence differences in the catalytic frame (amino acids *Fucus* 441-636). A major difference between the *Fucus*, *Ascophyllum* and *Corallina* algal bromoperoxidases and the fungal chloroperoxidases and various phosphatases is the additional basic amino acids in the first conserved domain of the bromoperoxidases, histidine at *Fucus* 464 and leucine at *Fucus* 472 for the brown algal enzymes, with threonine instead of leucine for the *Corallina* enzyme. These additional amino acids in the first conserved region are likely to be related to the greater activity of the bromoperoxidases with bromide, which is larger than the chloride ion.--

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 8, at the end of the application.